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Research Article

Development and Evaluation of Polyherbal Cream for Antiacne

S. Paragannavar*, A. Savadi, B. Pathan, R. Torgalmath, S. Chandaki, S. Rushikesh

Department of Pharmaceutics, Rani Chennamma College of Pharmacy, Belagavi-10, Karnataka, India

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ABSTRACT

Acne vulgaris is a prevalent chronic inflammatory disorder of the pilosebaceous unit that affects a significant proportion of the population, particularly adolescents and young adults. Its pathogenesis involves excessive sebum production, follicular hyperkeratinization, inflammation, and colonization by Propionibacterium acnes. The long-term use of synthetic anti-acne agents is often associated with adverse effects, prompting increased interest in herbal-based alternatives. In the present study, a polyherbal anti-acne cream was formulated using extracts of Ocimum sanctum (Tulsi), Azadirachta indica (Neem), and Triphala. The plant materials were extracted by the maceration method using ethanol as the solvent. Phytochemical screening confirmed the presence of bioactive constituents including alkaloids, flavonoids, glycosides, tannins, carbohydrates, and proteins. Two formulations (F1 and F2) were developed using the trituration method with beeswax, liquid paraffin, borax, methyl paraben, distilled water, and rose oil. Both formulations were evaluated for physicochemical and functional parameters such as organoleptic properties, pH, viscosity, spreadability, washability, phase separation, minimum inhibitory concentration (MIC), and zone of inhibition (ZOI). The formulations exhibited acceptable physicochemical characteristics and good stability. Notably, both F1 and F2 demonstrated significant antimicrobial activity against P. acnes. The findings suggest that the developed polyherbal cream is stable, safe, and effective, highlighting its potential as a natural therapeutic alternative for the management of acne vulgaris.

INTRODUCTION

Many people prefer the herbal product over the synthetic one, synthetic product produce several harmful side effects as compared to the herbal product have minimal side effect.[1]

Herbal cosmetics are defined as the beauty products which posses desirable physiological activity such as healing, smoothing, appearance, enhancing and conditioning properties of herbal ingredients. Nowadays there is great demand for the herbal cosmetics.[2]

***Corresponding Author:** S. Paragannavar
Address: *Department of Pharmaceutics, Rani Chennamma College of Pharmacy, Belagavi-10, Karnataka, India*
Email : sparagannavar@gmail.com
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The word "cosmetic" derived from a Greek word "kosmesticos" that means to adorn.[3]

TOPICAL DRUG DELIVERY

Over the last decades the treatment of illness have been accomplished by administrating drugs to human body via various routes namely oral, sublingual, rectal, parental, topical, inhalation. Topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorder or the cutaneous manifestations of a general disease with the intent of containing pharmacological or the effect of drug to the surface of the skin or within the skin semisolid formulations in all their diversity dominate the system for topical delivery.

Advantages of topical drug delivery system:

[1] Avoidance of first pass metabolism.

[2] Very slow absorption.

[3] Avoid of risk.

[4] Convenient and easy to apply.

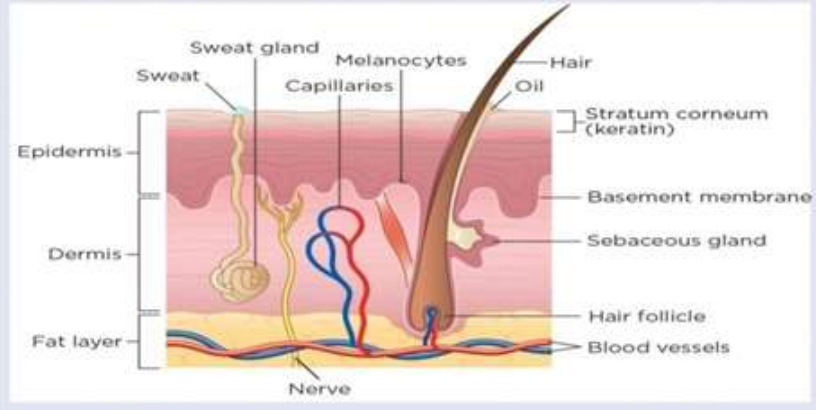
[5] Achievement of efficacy with lower total daily dosage of drug by continuous drug input.

[6] Avoid fluctuation of drug levels inter and intra patent variations.[3]

ANATOMY OF SKIN

In concern of weight and surface area, the skin is the largest organ in the body. Its surface area is around 16,000cm^2. Skin contributes to 8% of adult body weight. It is the live body's outermost layer or tissue. Skin behaves as a defense against the external environment. When disclose to sunshine, skin can generate a beneficial chemical compound known as Vit-D. The skin serves as a sensory organ and assists in controlling body temperature. Skin has a various types of biological components, including keratinocytes, melanocytes and erythrocytes. As a result of many components like cells and fibers, it exhibits multilayered structures. The skin consists of following skin layers.

Fig 1. **Cross-section through the skin**



A. The Epidermis:

The epidermis which has thickness of around 0.2mm. There are no veins and capillaries present in this layer. The thickness of epidermis varies on the location of the body. There are mainly 2 types of cells- keratinocytes and dendritic cells. It accommodates additional cells like melanocytes, Langerhans cells etc. Epidermis is also labeled as metabolic active tissues.

The outermost layer is categorized into 5 sub layers

1. Stratum corneum
2. Stratum lucidum
3. Stratum granulosum
4. Stratum spinosum
5. Stratum basale

B. The Dermis

Most of the dermis is made up of collagen and elastin as well as fibroblasts.

Its thickness is around 1-4mm. The dermis layer consists of two sublayers: papillary and reticular.

C. The Subcutaneous layer:

The deepest layer of the skin is the layer of fat that connects your bones to muscles. Its thickness is about 4-9mm. It goes so deep that the active ingredients in your skincare products can never reach. The subcutaneous layer is like a thermostat. It protects the body and can also be used as a source of energy in a pinch. Fat also acts as a filter, protecting your muscles, bones and organs from damage. Finally the subcutaneous layer contains additional blood vessels, nerve endings, hair follicular roots and the deepest oil – producing sebaceous glands. [4]

ACNE

The term Acne is derived from Greek word "Acme" which means "prime of life". Acne is a skin disorder that leads to an outbreak of lesions called pimples or "zits". Propionibacterium acnes and staphylococcus epidermidis are common pus – forming microbes responsible for the development of various forms of acne. The most common form of disease in adolescents is called acne vulgaris. Patients experience psychological burdens like depression, anxiety and low self-esteem because of acne. The introduction of novel herbal formulations for the treatment of acne may produce many advantages over previously used therapies. These herbal drugs are effective against a variety of Gram-positive and Gram-negative Bacteria. The concerned side effect of herbal drugs are much less compared with modern drugs. Thus, natural substances, which are obtained from the medicinal plant, having antibacterial and anti-inflammatory activity, are commonly employed for treatment of acne.

Types of Acne

- Acne Vulgaris
- Acne conglobata
- Acne Fulminans
- Gram Negative Folliculitis
- Acne Rosacea
- Pyoderma faciale . [5]

CREAM

Cream is defined as semisolid emulsions which are oil in water (o/w) or water in oil (w/o) type and these semisolid emulsions are intended for external applications. Cream is classified as oil in water and water in oil emulsions. It is applied on outer part or superficial part of the skin and its main ability is to remain for a longer period of time at the site of application. The functions of skin cream is to protect the skin against different environmental conditions, weather and gives soothing effect to the skin. There are different types of creams like cleansing, cold, foundation, vanishing, night, massage, hand and body creams.

Advantages of Herbal cream:

- Herbal cream includes natural ingredients.

- Natural products exhibit fewer side effects in contrast to synthetic counterparts.
- They have potential effectiveness in treating various skin issues.
- Herbal creams are generally considered to be more environment friendly compared to synthetic creams, as they are often biodegradable and do not contain harmful chemicals.[6]

AIM

The main aim of our work is to develop an herbal cream which can give multipurpose effect, like moisturizer, reduce acne and skin irritation and also adding glow to the face. We have used three herbal ingredients in our preparation which are Tulsi, Neem, and Triphala.

Tulsi is used to add glow to the skin and to promote wound healing and for antimicrobial property.

Neem is used as an antifungal and anti-inflammatory and it is also used to reduce scar, pigmentation, redness and itching of the skin.

Triphala possesses anti-fungal, anti-inflammatory, antimicrobial and wound healing property.[7][11]

TULSI

Tulsi is an aromatic shrub in the basil family Lamiaceae (tribe ocimeae) that is thought to have originated in north central India and now grows native throughout the eastern world tropics. Within Ayurveda, tulsi is known as "The Incompatible One", "Mother Medicine of Nature" and "The Queen of Herbs", and is revered as an "elixir of life" that is without equal for both its medicinal and spiritual properties.[8]

Tulsi extract shows inhibitory effects against pathogen such as Staphylococcus aureus, Propionibacterium acnes, Pseudomonas aeruginosa, E.coli, Klebsiella pneumoniae, Proteus Mirabilis, Salmonella typhimurium. The Tulsi leaves extract has some quantity of volatile oil which contain phytochemicals such as aldehyde, terpenes (sesquiterpenes, monoterpenes) and phenols and it also contains some quantity of saponins, taninns, glycosides, quinone, phlobatanin, flavonoids(orientin and vicenin), Steroids, coumarin and alkaloids. Tulsi is used in medicines and has various therapeutic properties and many useful phytochemicals which act as antimicrobial agents against pathogenic microbes.[9]

NEEM

Neem is a tree in the mahogany family Meliacea. Neem extracts are found to be antimicrobial, antifungal, antiviral, antibacterial and antidiabetic. The chemical constituents and phytoconstituents of Neem are biologically active. Compounds may include secondary metabolites like flavonoids, steroids, tannins, terpenoids, saponins in varying concentrations. Azadirachtin, terpenoid is a low toxic compounds. An antimicrobial is an agent, which kills or inhibit the growth of microorganisms. Neem shows antimicrobial activity against some microorganisms.[10]

TRIPHALA

Triphala is a well-known polyherbal formulation from Ayurveda. It is a rasayana Drug in Indian System of Medicine (ISM). Triphala is a mixture of three fruits which is composed of dried fruits of Embelica offincinalis Gaertn (Euphorbiaceae), Terminalia belerica Linn (Combertaceae) and Terminalia chebula (Combertaceae) in equal proportion (1:1:1) as described in Ayurvedic Formulary of India. It is also used as blood purifier

that can improve mental faculties and posseses anti-inflammatory, analgesic,hypoglycemic and anti-aging properties. Triphala is claimed to have antiviral and antibacterial effects. Triphala inhibits the growth of Gram positive and Gram negative bacteria. Triphala is rich in Gallic acid, Vitamin - C, ellagic acid, chebulic acid, bellericanin and ß-sitosterol. Triphala Mashi exhibit antimicrobial activity attributed to phenolic compounds and tannins in triphala. Triphala churna has antibacterial activity against various bacterial pathogens.[11]

MATERIALS AND METHODOLOGY

The active ingredients and other excipients which were used in the present study was given in the table 1 along with their source, grade etc.

Table 1 : List of chemicals used with their grade and sources.

Serial no.	Materials	Supplier
1	Tulsi	Kle Ayurvedic Aushadhalay
2	Neem	Kle Ayurvedic Aushadhalay
3	Triphala	Kle Ayurvedic Aushadhalay
4	Bees wax	RCCP
5	Borax	RCCP
6	Methyl paraben	RCCP
7	Glycerin	RCCP
8	Rose oil	RCCP

Extraction of plant materials using cold maceration method:-

Tulsi:-[12]

- Green and fresh Tulsi was collected from the plant.
- The leaves were cleaned by distilled water and then leaves were separated from the branches manually.
- The leaves were allowed for air drying under room temperature.
- The dried leaves were blended to a powder by grinder and stored in air tight glass container until required for preparation.
- The 50 gm of crushed raw material was subjected to maceration with 200 ml of absolute ethanol in beaker and sealed with aluminum foil and kept in the dark for 7 days.
- The beaker was stirred to ensure uniform and complete extraction.
- The mixture was filtered by using clean muslin cloth and the filtrate was collected in a cleansed beaker.
- The filtrate is concentrated using rotary evaporator and then air dried.

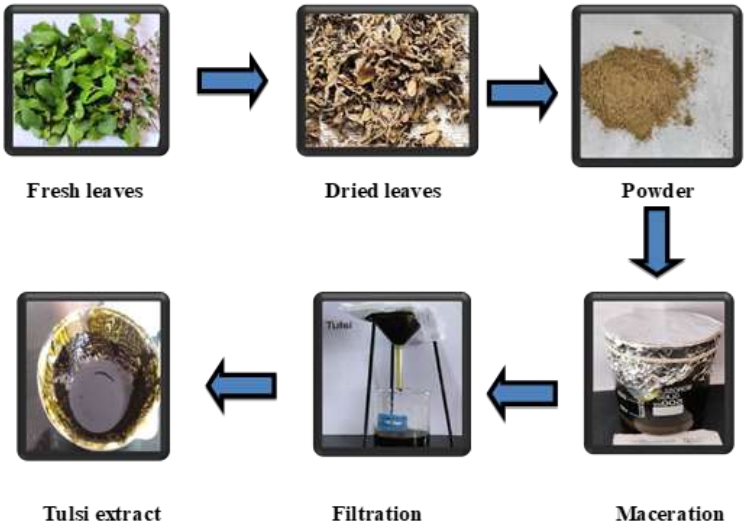


FIGURE 1: MACERATION OF TULSI

Neem:- [13]

- The fresh leaves were collected, and washed in a tap water, rinsed in a sterile distilled water and dried for 10 days under room temperature.
- The dried leaves were then blended to powder a clean grinder and stored in an air tight glass containers until required for preparation.
- The 50 gm of leaves of Neemwere weighted into beaker and 200 ml of ethanol were added and left to extract at room temperature for 5 days.
- The mixture was filtered by using clean muslin cloth and the filtrate was collected in a cleansed beaker.
- The filtrate is concentrated using rotary evaporator and then air dried
- The filtrate is concentrated using rotary evaporator and then air dried.

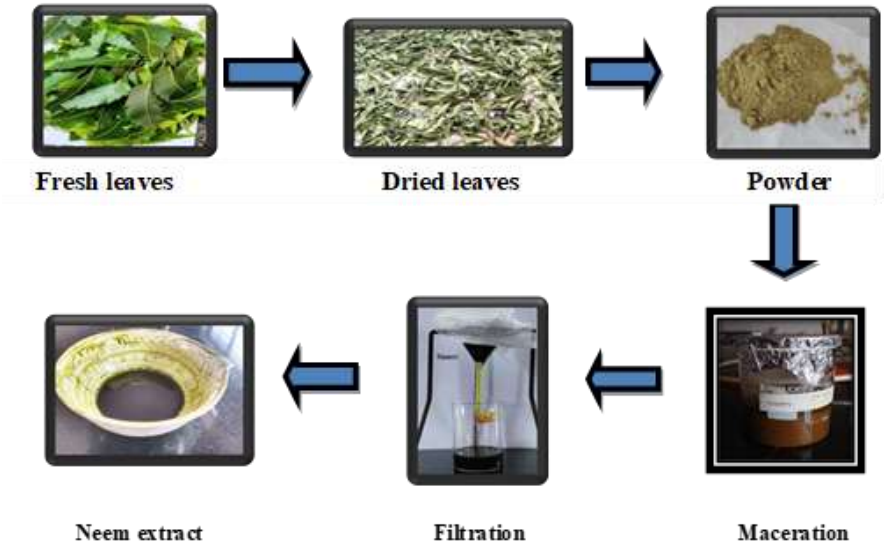


FIGURE 2: MACERATION OF NEEM

Triphala:-[14]

- The dried three fruits, Triphala contains Indian gooseberry (*Emblica officinalis*), Black myrobalan (*Terminalis chebula*), Belleric myrobalan (*Terminalis belerica*)
- The ethanolic extract of Triphala was produced, the powder was macerated in 95% ethanol for 7 days, then filtered through Whatman no.4 paper.
- The supernatant was evaporated under rotary evaporator.
- The extract was kept at 4^0C until used.

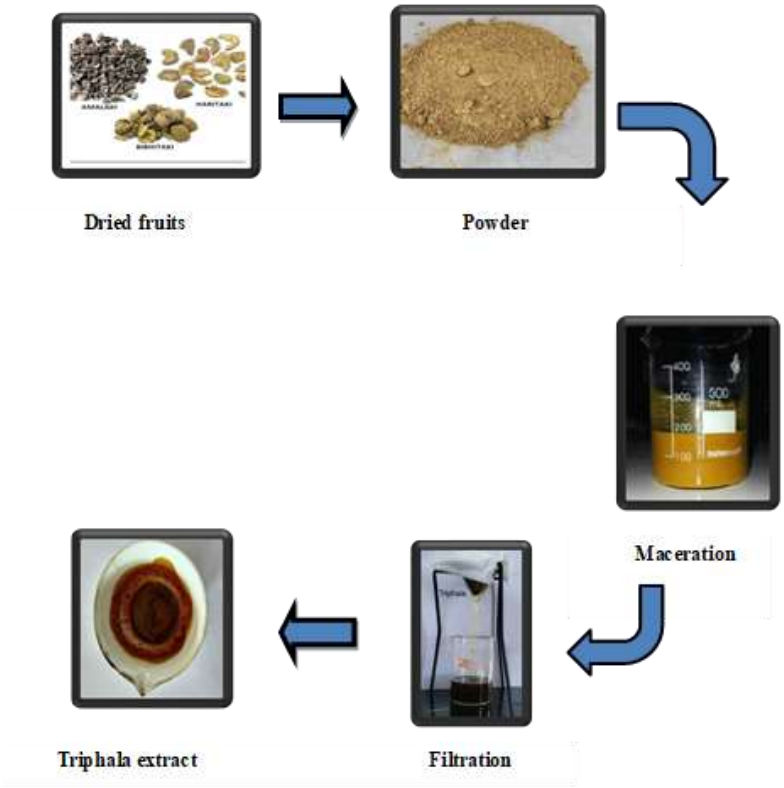


FIGURE 3: MACERATION OF TRIPHALA

Preliminary phytochemical screening

- **Test for Carbohydrates:**

1. **Molisch test:** To 2-3 ml aqueous extract add few drops of alpha- naphthol solution in alcohol shake and add conc.H_2SO_4 from sides of the test tube violet ring is formed at the junction of two liquids.
2. **Fehling's test:** mix 1ml Fehling's A and 1ml of Fehling's B solutions, boil for 1 min. Add equal volume of test solution. Heat in boiling water bath for 5-10 min. First a yellow, then a brick red color is observed.

- **Test for Alkaloids:**

1. **Dragendroff's test:** To 2-3 ml filtrate add few drops of Dragendroff's reagent. Orange brown precipitate is formed.
2. **Wagner's test:** To 2-3 ml of filtrate add few drops of wagner's reagent,gives reddish brown precipitate.

- **Test for Flavonoids:**

1. **Shinoda test:** To dry powder or extract, add 5ml. 95% ethanol,few drops conc. HCl and 0.5gm magnesium turnings. Pink color observed.

2. **Lead acetate test:**To small quantity of residue, add lead acetate solution. Yellow colored precipitate is formed.

- **Test for glycosides:**

1. **Keller killani test:** To 2ml extract, add glacial acetic acid, add 1 drop of 5%$Fecl_3$ and conc.H_2SO_4 reddish brown color appears at the junction of two liquid layers and upper layer appears bluish green.

2. **Borntrager's test:** To 3ml extract,add dil.H_2SO_4 . Boil and filter. To cold filter, add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia, ammonical layer turns pink or red.

- **Test for Tannins:**

1. **Gelatin test:** To 2-3 ml aqueous or alcoholic extract, add few drops of gelatin reagent white precipitate observed.

2. **5% $FeCl_3$ test:** To 2-3 ml of aqueous or alcoholic extract, add few drops of 5% $FeCl_3$solution deep blue-black color observed.

- **Test for proteins :**

1. **Millon's test:** Mix 3ml test sample with 5ml Millon's reagent. White ppt. warm ppt turns brick red or the ppt dissolves giving red colored solution.

2. **Lead acetate:** The test solution gives white colloidal ppt with 5% lead acetate solution.

PREPARATION OF ANTI ACNE CREAM

1. Use 2 borosilicate china dish. Add liquid paraffin and beeswax in one china dish, and heat the mixture to 75^0C [oil phase].
2. Take borax, glycerin and distilled water in another china dish and heat to a temperature of 75^0C [Aqueous phase].
3. Slowly add aqueous phase to oil phase.
4. Stir it vigorously until it forms a smooth cream. Now add drug extracts, rose oil and methyl paraben and triturate thoroughly for uniform mixing.
5. Store the cream in an air tight container.

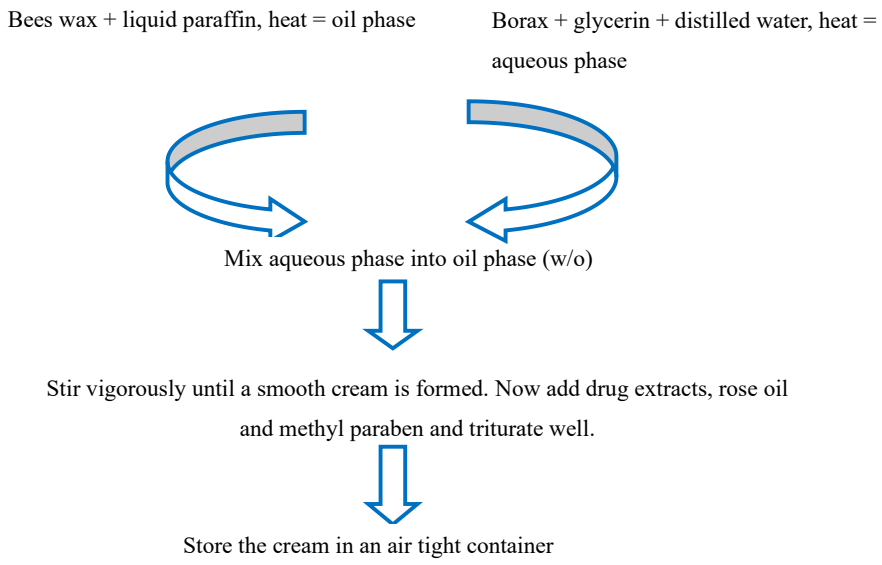


FIGURE 4: FLOWCHART FOR PREPARATION OF CREAM

Table no.2 Formulation of polyherbal anti acne cream

Sr . No	Ingredients	F1	F2	Category
1	Neem extract	1g	1g	API
2	Tulsi extract	1g	1g	API
3	Triphala extract	1g	1g	API
4	Bees wax	3g	4g	Emulsifying agent
5	Liquid paraffin	10g	10g	Soothing agent
6	Borax	0.2g	0.2g	Emulsifying agent
7	Glycerine	1g	1g	Moisturizer
8	Methyl paraben	0.02g	0.03g	Preservative
9	Rose oil	q.s	q.s	Fragrance
10	Distilled water	5.61g	5.61g	Vehicle

FIGURE 5: Formulation 1 & 2

POST FORMULATION STUDIES:-[1][14]

Evaluation parameter

1) Physical evaluation:-

The physical parameters of cream like color, odor, consistency, and state of formulations analyzed.

- The color of the formulation was checked against white background.
- Consistency- The consistency was checked by applying on the skin.
- Greasiness- The greasiness was assessed by the application on to the skin.
- Odor- The odor of the cream was checked by mixing the cream in water and taking the smell.

2) Washability:-

On the hand, a little amount of cream was applied and washed it with tap water.

3) Phase separation :-

Generally, this test is checked every 24 to 30 hours. Put the cream into the container at room temperature and protect the formulation from the light for this.

4) pH determination :-

The pH of the cream was determined using digital pH meter. The readings were taken for average of 3 times.

5) Spreadability :-

Spreadability of formulations was determined by an apparatus suggested by Multimer et al.which was fabricated in laboratory and used for study.

Procedure: An excess of cream sample 2.5g was placed between two glass slides and a 1000g weight was placed on slides for 5 minutes to compress the sample to a uniform thickness. weight (60g) was added to pan. The time (seconds) required to separate the two slides was taken as a measure of spreadability.

It was calculated using the formula

$$S = m.l / t$$

Where s – spreadability in g.cm / sec

m- weight tied on upper side(60g)

l – length of glass slideMG

t – time in seconds

6) Viscosity:-

Viscosity of the cream was measured by using Brookfield viscometer. DVII model with a T-bar spindle in combination with a helipath stand T 95 was used for the measurement of viscosity of all gels. The viscosity was measured using 50 gm of cream filled in 100 ml beaker. The T-bar spindle (T 95) was lowered perpendicular in the center taking care that spindle does not touch the bottom of jar.

Measurement of viscosity

The T-bar spindle (T95) was used for determining the viscosity of the creams. The factors like temperature, pressure and sample size etc. which affect the viscosity was maintained constant during the process. The helipath T-bar spindle was moved up and down giving viscosities at number of points along the path. The torque reading was always greater than 10%. The average of three readings taken in one minute was noted as the viscosity of creams.

7) Anti-microbial study of extracts:-

- Assessment of MIC of Tulsi, Neem and Triphala
- Bacterial culture: Propionobacterium acnes.
- Method used: Minimum inhibitory concentration and zone of inhibition.

A) Determination of MIC:

1. The stock solution of sample was prepared in dimethyl sulfoxide (DMSO).
2. Two fold serial dilution of sample in BHI broth were carried out with concentration ranging from 100µg/ml to 600µg/ml.
3. 100 microliters of an earlier prepared *P.acne* strain was added to all the MIC tubes.
4. The tubes were incubated at 37^0C for 48 hours in an anaerobic condition. After the incubation the bacterial growth was recorded visually.
5. Further to 100µl of incubated growth add 30µl of Resazurin dye and incubated for 4 hours at 37^0C.Based on the color change in the well growth of bacteria was noted.
6. The lowest concentration at which no bacterial growth was found was considered as the MIC value.

B) Zone of inhibition:

Agar well diffusion method:

1. BHI agar plates incubated with the test organism and with cream taken as sample and loaded into the wells of the agar.

2. All plates were incubated (37^0C) for 24 hours.After incubation the diameters of any clear zones around the anti-microbial containing discs or wells were measured using scale.

RESULTSAND DISCUSSION:

PREFORMULATION STUDIES:

Extraction: Tulsi, Neem, and Triphala all have good ability to extract solvent in ethanol. Maceration process gives better yield in all three plants used.

Phytochemical screening: Table 3 represents a phytochemical screening of the ethanolic extract of Tulsi, Neem and Triphala.

Table no.3 Results of phytochemical screening of drug extracts

Sr. No.	Phytoconstituents	Name of test	Tulsi	Neem	Triphala
1	Carbohydrates	Molisch test	+	+	-
		Fehling's test	+	+	-
2	Alkaloids	Dragendorff's test	+	+	+
		Wagner's test	+	+	+
3	Flavonoids	Shinoda test	+	+	+
		Lead acetate test	+	+	+
4	Glycosides	Keller killani test	+	+	+
		Borntragers test	+	+	+
5	Tannins	Gelatin test	+	+	+
		5% $FeCl_3$ test	+	+	+
6	Proteins	Millon's test	-	-	+
		Lead acetate test	-	-	+

PHYTOCHEMICAL SCREENING OF TULSI

FIGURE 6: PHYTOCHEMICAL SCREENING OF TULSI

PHYTOCHEMICAL SCREENING OF NEEM

FIGURE 7: PHYTOCHEMICAL SCREENING OF NEEM

PHYTOCHEMICAL SCREENING OF TRIPHALA

FIGURE 8: PHYTOCHEMICAL SCREENING OF TRIPHALA

POST FORMUION STUDIES:

Table no.4 Results of evaluation parameters of anti acne cream

Sr.no	Evaluation test	Formulation 1[F1]	Formulation 2[F2]	
1	Physical evaluation			
	color	Tea green	Tea green	
	Texture	Smooth	Smooth	
	State	Semi solid	Semi solid	
2	Washability	Easily washable	Easily washable	
3	Phase separation	No phase separation	No phase separation	
4	pH	6	7	
5	Spreadabilty(g/sec)	35	40	
6	Viscosity(cps)	21020	11810	
7	Anti- microbial test	MIC = 600µg		
		ZOI	3mg	8.11mm
			4mg	11.44mm

4) pH :

Table no.5 Results of pH

Formulation	pH reading
F1	6
F2	7

FIGURE 9: **Formulation 1** **Formulation**

5) Spreadability :

Table no.6 Results of spreadability

Formulation	Reading (gm/sec)
F1	35
F2	40

FIGURE 10: **Formulation 1** **Formulation 2**

6)Viscosity:

Table no.7 Results of viscosity

Formulation	Reading(cps)
F1	21020
F2	11810

FIGURE 11: Formulation 1 Formulation 2

7) Anti-microbial test :

Results:

Antibacterial activity by Agar well diffusion method:

Table 1: antibacterial properties of samples in Zone of inhibition by agar well diffusion

Sl.	Sample Name	Conc. (mg)	*Propionibacterium acnes*
1	Sample	1	—
		2	—
		3	8.11mm
		4	11.44mm

Antibacterial activity by broth dilution method:

Table 2: Antibacterial properties of samples in MIC

Sl.	Sample name	*Propionibacterium acnes*
		MIC
1	Cream	600 µg
2	Kanamycin	10 µg

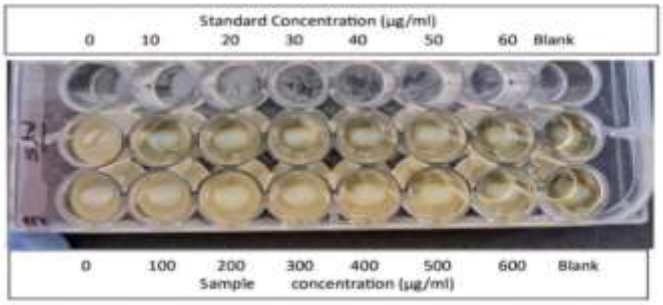


FIGURE 21: MIC test of sample

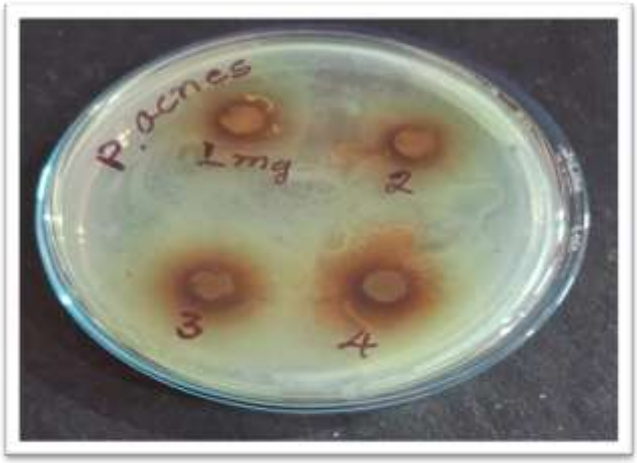


FIGURE 22: Zone of inhibition of test sample.

SUMMARY

Polyherbal anti acne cream containing natural ingredients was developed and evaluated using various parameters. Tulsi, Neem& Triphala were extracted by ethanol using maceration method.

These extracts were characterized by various chemical tests like test for alkaloids, carbohydrates, flavonoids, glycosides, tannins, proteins etc. Further, the anti acne cream was prepared by using other ingredients such as bees wax, borax, liquid paraffin, methyl paraben, glycerin, rose oil and distilled water.

The developed formulation was evaluated for parameters like pH, spreadability, washability, viscosity, phase separation, irritancy, and anti – microbial activity, the results obtained of the developed cream was found to be satisfactory.

CONCLUSION:

We made a prompt attempt to develop polyherbal anti acne cream containing Tulsi, Neem, Triphala. The cream was evaluated for various physical and microbiological evaluations.

From the result it was concluded that the anti acne cream was found to be effective against P.acne.

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